OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

Mag-Bind® Normalizer Kit

M6422-00	1 x 96 preps
M6422-01	4 x 96 preps
M6422-02	20 x 96 preps

November 2012

For research use only. Not intended for diagnostic testing.

Mag-Bind® Normalizer Kit

Table of Contents

Introduction and Overview.....2
Kit Contents/Storage and Stability.....3
Preparing Reagents.....4
DNA in Various Elution Buffers.....5
DNA in Water.....8
Troubleshooting Guide.....11
Ordering.....12

Manual Revision: November 2012

OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction

Many high-throughput applications, such as sequencing and genotyping, require the input DNA concentration to be within a certain range for optimal results. However, DNA prepared from multiple sources or extracted from different batches very often will have considerable variability in quantity and quality. Traditionally, a tedious process of quantification, calculation and concentration adjustment must be carried out to normalize the DNA samples.

The Mag-Bind® Normalizer Kit completely eliminates the need to quantify and aliquot DNA, saving time and tip cost. Using our proprietary Mag-Bind® Normalizer Beads and binding buffer system, input DNA of various quantities is simply bound, washed and eluted to a final normalized product. The magnetic beads have a limited binding capacity and therefore allow a predefined amount of DNA to be captured and eluted. Additionally, the binding ability is proportional to the amount of beads used, so users can make easy adjustment to suit their downstream application needs. The Mag-Bind® Normalizer Kit is fully automatable on multiple liquid handling platforms including Hamilton STAR, Tecan Evo, Caliper Sciclone, and Beckman Coulter Biomek instruments.

Binding Capacity:

5 µL Mag-Bind® Normalizer Beads can bind 200 ng PGEM plasmid DNA.

Kit Contents

Product	M6422-00	M6422-01	M6422-02
Preparations	1 x 96	4 x 96	20 x 96
Mag-Bind® Normalizer Beads	1.1 mL	4.5 mL	22 mL
Normalizer Binding Buffer	20 mL	80 mL	400 mL
NAC Buffer	600 µL	3 mL	12 mL
SPM Wash Buffer	15 mL	60 mL	4 x 60 mL
Elution Buffer	10 mL	40 mL	200 mL
User Manual	✓	✓	✓

Storage and Stability

All Mag-Bind® Normalizer Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Normalizer Beads must be stored at 2-8°C. All other components should be stored at room temperature. If any precipitates form in the buffers, warm at 37°C to dissolve.

- Dilute SPM Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M6422-00	35 mL
M6422-01	140 mL
M6422-02	140 mL per bottle

Mag-Bind® Normalizer Kit Protocol - DNA in Various Elution Buffers

Materials and Equipment to be Supplied by User:

- Magnetic Separation Device (Recommended ALPAQUA® A001322)
- Vortexer
- Sealing Film
- 100% ethanol
- 96-well PCR plate
- Nuclease-free water

Before Starting:

- Prepare SPM Wash Buffer according to Preparing Reagents section on Page 4.

1. Add 50 µL sample a 96-well PCR plate.

   For best results, the initial DNA input amount should be 3X the desired output amount. Decreased DNA input amounts will cause variability in the normalized DNA.

2. Add 5 µL NAC Buffer, 100 µL 100% ethanol, and 10 µL Mag-Bind® Normalizer Beads.

   Note: Every 10 µL Mag-Bind® Normalizer Beads can bind 200 ng PGEM plasmid DNA. Increasing the amount of Mag-Bind® Normalizer Beads has a linear effect on the amount of product isolated. The lowest elution volume that can be used is 20 µL. If the desired concentration is >50 ng/µL, increase the amount of Mag-Bind® Normalizer Beads used per each well.

3. Vortex or pipet up and down to mix thoroughly.

4. Let sit at room temperature for 5 minutes.

5. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Normalizer Beads. Let sit at room temperature until the Mag-Bind® Normalizer Beads are completely cleared from solution.

6. Aspirate and discard the supernatant.

7. Remove the plate from the magnetic separation device.

8. Add 50 µL nuclease-free water. Vortex or pipet up and down to mix thoroughly.

9. Add 50 µL Normalizer Binding Buffer. Vortex or pipet up and down to mix thoroughly.

10. Let sit at room temperature for 5 minutes.

11. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Normalizer Beads. Let sit at room temperature until the Mag-Bind® Normalizer Beads are completely cleared from solution.

12. Aspirate and discard the supernatant.

13. Remove the plate from the magnetic separation device.

14. Add 100 µL Normalizer Binding Buffer. Vortex or pipet up and down to mix thoroughly.

15. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Normalizer Beads. Let sit at room temperature until the Mag-Bind® Normalizer Beads are completely cleared from solution.

16. Aspirate and discard the supernatant.

17. Add 150 µL SPM Wash Buffer. Pipet up and down to mix while leaving the plate on the stand.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

18. Let sit at room temperature for 1 minute on the magnetic separation device.

19. Aspirate and discard the supernatant. Leave the plate on the magnetic separation device.

20. Repeat Steps 17-19 for a second SPM Wash Buffer wash step. Make sure that all liquid is completely removed before continuing to Step 21.

21. Let sit at room temperature for 5 minutes with the plate on the magnetic separation device to dry the Mag-Bind® Normalizer Beads.

22. Remove the 96-well plate from the magnetic separation device.

23. Add 20-100 µL Elution Buffer. Adjust the volume of elution buffer to achieve the desired concentration of the final product.

    Note: Every 10 µL Mag-Bind® Normalizer Beads can bind 200 ng PGEM plasmid DNA. Increasing the amount of Mag-Bind® Normalizer Beads has a linear effect on the amount of product isolated. If the desired concentration is 5 ng/µL, use 40 µL Elution Buffer.

24. Vortex or pipet up and down to mix thoroughly.

25. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Normalizer Beads. Let sit at room temperature until the Mag-Bind® Normalizer Beads are completely cleared from solution.

26. Aspirate and transfer the supernatant containing the purified DNA to a new 96-well plate.

27. Store the DNA at -20°C.

Mag-Bind® Normalizer Kit Protocol - DNA in Water

Materials and Equipment to be Supplied by User:

- Magnetic Separation Device (Recommended ALPAQUA® A001322)
- Vortexer
- Sealing Film
- 100% ethanol
- 96-well PCR plate

Before Starting:

- Prepare SPM Wash Buffer according to Preparing Reagents section on Page 4.

1. Transfer 50 µL sample to a 96-well PCR plate.

2. Add 50 µL Normalizer Binding Buffer and 10 µL Mag-Bind® Normalizer Beads. Vortex or pipet up and down to mix thoroughly.

   Note: Every 10 µL Mag-Bind® Normalizer Beads can bind 200 ng PGEM plasmid DNA. Increasing the amount of Mag-Bind® Normalizer Beads has a linear effect on the amount of product isolated. The lowest elution volume that can be used is 20 µL. If the desired concentration is >50 ng/µL, increase the amount of Mag-Bind® Normalizer Beads used per each well.

3. Let sit at room temperature for 5 minutes.

4. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Normalizer Beads. Wait until all the magnetic particles have completely migrated to the wall of the wells adjacent to the magnet.

5. Aspirate and discard the supernatant.

6. Remove the plate from the magnetic separation device.

7. Add 100 µL Normalizer Binding Buffer. Vortex or pipet up and down to mix thoroughly.

8. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Normalizer Beads. Let sit at room temperature until the Mag-Bind® Normalizer Beads are completely cleared from solution.

9. Aspirate and discard the supernatant. Leave the plate on the magnetic separation device.

10. Add 150 µL SPM Wash Buffer. Pipet up and down to mix while leaving the plate on the stand.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

11. Let sit at room temperature for 1 minute on the magnetic separation device.

12. Aspirate and discard the supernatant. Leave the plate on the magnetic separation device.

13. Repeat Steps 10-12 for a second SPM Wash Buffer wash step. Make sure that all liquid is completely removed before continuing to Step 14.

14. Let sit at room temperature for 5 minutes with the plate on the magnetic separation device to dry the Mag-Bind® Normalizer Beads.

15. Remove the 96-well plate from the magnetic seapartion stand.

16. Add 20-100 µL Elution Buffer. Adjust the volume of elution buffer to achieve the desired concentration of the final product.

    Note: Every 10 µL Mag-Bind® Normalizer Beads can bind 200 ng PGEM plasmid DNA. Increasing the amount of Mag-Bind® Normalizer Beads has a linear effect on the amount of product isolated. If the desired concentration is 5 ng/µL, use 40 µL Elution Buffer.

17. Vortex or pipet up and down to mix thoroughly.

18. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Normalizer Beads. Let sit at room temperature until the Mag-Bind® Normalizer Beads are completely cleared from solution.

19. Aspirate and transfer the supernatant containing the purified DNA to a new 96-well plate.

20. Store the DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Low DNA Yields	
Elution Using Water: Water pH is too low (<7.5)	Check the pH of the water, adjust the pH of the water to 8.0 using Tris-HCl (2M, pH 8.5).
Low Input DNA	Use at least 3X amount of starting volume to achieve desired results.
No DNA eluted	
SPM Wash Buffer was not diluted with 100% ethanol	Prepare SPM Wash Buffer as instructed on the bottle, or refer to Page 4.
Optical densities do not agree with DNA yield on agarose gel	
Trace contaminants eluted from Mag-Bind® Normalizer Beads increase A_{260}	Rely on agarose gel/ethidium bromide electrophoresis for quantification.
DNA sample floats out of well while loading agarose gel.	
Ethanol was not completely removed from the Mag-Bind® Normalizer Beads	Completely remove any residual ethanol at Step 20 (elution buffer) or Step 13 (water). Increase the drying time in Step 21 (elution buffer) or Step 14 (water).

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Elution Buffer (100 mL)	PDR048
SPM Wash Buffer (40 mL)	PS014
2 mL Collection Tubes	SS1-1370-00
1.5 mL DNase/RNase-free Microcentrifuge Tubes	SS1-1210-0

HiBind®, E.Z.N.A.®, and MicroElute® are registered trademarks of Omega Bio-tek, Inc.
PCR is a patented process of Hoffman-La Roche. Use of the PCR process requires a license.